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Biomarker Responses to Environmental Stressors in the Hairy Mangrove Crab, *Sesarma huzardii* (Graspidae) from a Tropical Lagoon Mudflat in Nigeria

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ABSTRACT

The study evaluated antioxidant and oxidative stress responses in the Hairy Mangrove Crab S*esarma huzadii* inhabiting contaminated nearshore locations in Lagos lagoon mudflats through the catalase enzyme activity (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) and Thiobarbituric Acid Reactive Substances (MDA/TBARS). The results indicates that obtained metal levels differed significantly ($P < 0.05$) across stations, with relatively higher mean values recorded at locations closest to domestic and solid waste dumps. Estimated Bio-sediment accumulation factor was highest for Copper in the tissues of *S. huzadii*. Relatively low levels of SOD activities were recorded in crabs from Abule-Agege and Okobaba suggests potential stress from the locations. Significant differences were also observed in anti-oxidative stress enzyme activities and lipid peroxidation levels in tissues of *S. huzadii* across sites. Obtained levels of antioxidant enzymes and MDA in *S. huzadii* further indicates increasing level of environmental stressors in the areaHowever, the biochemical responses of biota to the interactions between season, contaminant level, multiple stressors or complex environmental settings needs to be fully understood and taken into consideration through continuous monitoring programs..

1. INTRODUCTION

Mangrove ecosystems fringe tropical and sub-tropical coastlines throughout the world and function as nurseries for a wide variety of vertebrate and invertebrate marine species (Olafsson et al., 2002). The Hairy Mangrove Crab, *Sesarma huzardi* are amphibious in habit and can be found round intertidal areas with moist/wet muddier regions of the mangrove (Lawal-Are and Nwankwo, 2011). Although the mangrove crab does not really constitute a food item for the coastal communities, they however play a major ecological role in the mangrove ecosystem through their feeding on the fallen leaves. Mangrove crabs have been found in swamps of the Lagos Lagoon, distributed up to the tidal limit in the lagoon. Over the years, several studies have emphasized the enormous threats posed to ecological receptors within the Lagoon and other interconnected ecosystems (Chukwu, 2006; Usese et al., 2017a).

Biomarker responses are generally considered to be intermediates between pollutant sources and higher-level effects (Suter, 1990; Anagboso et al., 2010). Chemical biomarkers such as antioxidant enzymes and evidence of oxidative damage to bio molecules are powerful tools for detecting the exposure and biological effects of pollutants, allowing early

detection of environmental problems (Bouraoui et al., 2010). The relationship between exposure to toxicants and enzymes such as catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione-S-transferase (GST) as well as lipid peroxidation products has been the subject of several investigations (Walker, 1998). Catalase activity is also frequently employed as a biomarker and biomonitoring tool for the evaluation of oxidative status of aquatic animals exposed to polluted waters. The application of chemical biomarkers under field conditions has been proposed by many investigators to assess chronic responses and to address the integrated effects of anthropogenic and environmental stressors.

A number of researchers have reported the effects of exposure to varying concentrations of pollutants on lipid peroxidation and antioxidant status in crab (Vijayavel et al., 2004; Martin Diaz et al., 2005; Anagboso et al., 2009). However, there is paucity of information on the oxidative stress and antioxidant responses in species of mangrove crab found in Nigeria. The main thrust of the present study was to use chemical biomarkers to evaluate the health status of crabs inhabiting contaminated locations in the mangrove swamp of Lagos Lagoon.

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2. MATERIALS AND METHODS

2.1 Study Site

The sampling area, located between latitudes 6º 26' – 37' N and longitude 3º 23 – 4º 20'E on the highly populated western axis of the Lagos Lagoon is fringed by mangrove swamps (Moruf and Lawal-Are, 2015). Site selection (Fig. 1) was based on increasing anthropogenic effects from heaps of domestic and solid waste dumps

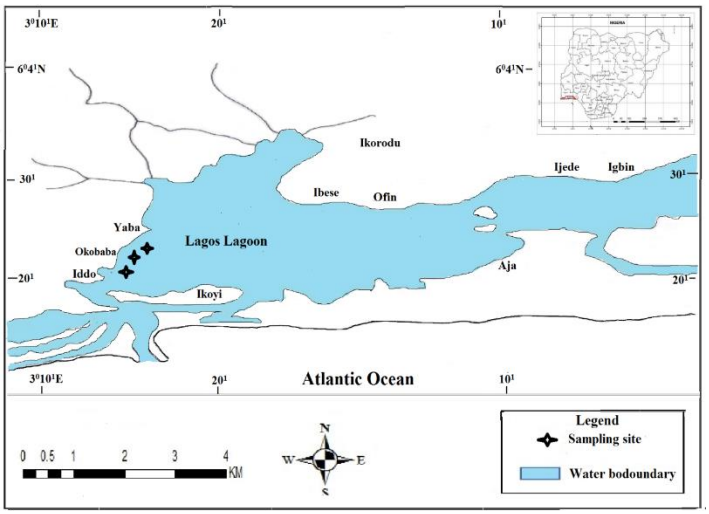

Figure 1: Map of the Lagos Lagoon showing sampling sites

2.2 Collection of Samples

The test animal (Plate 1), *S. huzardii* with length which ranged from 2.6cm to 4.0cm and weight from 4.9g to 29.0g were randomly handpicked from the sites where they were attached to wood or buried in mud just below the surface. Crabs were immediately placed in styrofoam boxes without water and were transported to the Aquatic Toxicology and Ecophysiology laboratory at the Department of Marine Sciences, University of Lagos for further processing.

2.3 Laboratory Analysis

2.3.1 Heavy metal levels in sediment and tissues of *Sesarma huzardii*

In order to determine heavy metal levels, air dried sediment samples were sieved through a 200 µm merge and the uniform particles were then digested using established technique (Don-Pedro et al., 2004) while the muscle tissues of *S. huzardii* were oven dried at 70°C for 1 hr and ground to powder in ceramic mortars and 0.5 g of each sample were made into paste by adding double distilled water. This was followed by digestion using 5 ml of 1 M HNO_3 and mild heat until brown fumes appeared.

Plate 1: Hairy Mangrove Crab, *Sesarma huzardii* (Decapoda: Graspidae)

The samples were cooled off, made up to 50 ml in a standard volumetric flask which have been subjected to acid wash to remove any trace of residual metals and then filtered prior to analysis. Thereafter, processed samples were analysed using Atomic Absorption Spectrophotometer (Perkin Elmer series) to determine levels of selected heavy metals.

The biota to sediment accumulation factor (BSAF) was determined using the method of Thomann et al., (1995)

$$BAF = \frac{\text{Heavy metal concentration in Flesh}}{\text{Heavy metal concentration in Sediment}} \ldots\ldots\ldots$$

Equation IX

2.3.2 Evaluation of Responses

Samples of excised muscle tissues of crabs stored at −20 °C were later thawed and homogenized for the assays of reduced glutathione, catalase, superoxide dismutase, levels of proteins, and lipid peroxidation (TBARS) following the protocol described by Lushchaks et al., (2005) and Bertholdo-Vargas et al., (2009). Lipid peroxidation estimation was carried out through the determination of thiobarbituric acid reactive substance (TBARS) which are indices of membrane lipid peroxidation.

2.4 Statistical Analysis

Statistical analyses were carried out using SPSS version 17. The biochemical results and antioxidant enzyme activity data were subjected to one-way analysis of variance (ANOVA) and significant difference were determined at 5% confidence level ($P<0.05$). Post-hoc comparisons of means between stations were carried out using Student Newman Keuls (SNK) test to determine which values differed significantly.

3. RESULTS

3.1 Heavy metal concentrations in sediment and the tissues of *S. huzardii*

Almost all examined heavy metals (Cd, Cu, Fe, Mn, Zn and Pb) were observed to accumulate in varying and sometimes very low but measurable concentrations amongst samples and across sites (Table 1). Generally, metal levels differed significantly ($P<0.05$) with highest mean concentrations recorded at locations which lie closest to domestic and solid waste dumps. Cadmium (Cd) with a mean concentration in the sediment varying from below detection limit (BDL) to 0.04 mg/kg was only detected at two of the sampling locations (Okobaba and site adjoining Abule-Agege creek). Similarly, the concentration of Pb in the sediment samples varied from below detection limit to 0.25mg/kg (Abule-Agege). Pb with mean concentration of 0.001 mg/kg and BSAF of 0.004 were only recorded in tissues of crab from the study site adjoining Abule-Agege creek. Copper (Cu), iron (Fe) zinc (Zn) and Manganese (Mn) were detected in sediment and tissues of *S. huzardii* in all sample locations. Highest mean concentration of Cu was recorded at Abule-Agege for sediment and tissues of crab while the lowest was recorded at Yaba (University of Lagos water front) for sediment and crab tissue respectively. The results also showed that the estimated bio-sediment accumulation factor (BSAF) was highest for Cu at Yaba (1.40) while the lowest BSAF of 0.74 was obtained in the tissues of *S. huzardii* at Abule-Agege. Fe concentration in sediment and tissue also varied significantly ($P<0.05$) between sampling sites with highest metal concentrations and BSAF recorded for samples from Abule-Agege and lowest at Yaba. Zn and Mn levels followed a similar trend with highest concentrations observed at Abule-Agege in sediment and crab tissues.

Table 1: Mean Heavy metal concentrations (mg/kg) in Sediments and *S. huzardii* tissue with biota to sediment accumulation factor (BSAF)

Sampling location	Cd	Cu	Fe	Mn	Zn	Pb
Yaba (Unilag Lagoon front)						
Sediment	BDL	2.31	27.56	0.83	7.41	BDL
Tissue	BDL	3.24	12.68	0.36	5.30	BDL
BSAF	*	1.40	0.46	0.43	0.72	*
Okobaba						
Sediment	0.02	2.90	88.02	0.72	10.43	0.003
Tissue	BDL	3.84	48.57	0.03	4.86	BDL
BSAF	*	1.32	0.55	0.04	0.47	*
Agbule-Agege (VC'slodge)						
Sediment	0.04	9.66	105.11	2.78	20.81	0.25
Tissue	BDL	7.12	68.79	1.84	9.28	0.001
BSAF	*	0.74	0.65	0.66	0.45	0.004

BDL =below detection limit; * = Data not available

Table 2: Lipid peroxidation and Antioxidant enzyme activity in tissues of *S. huzardii*

Biomarkers	Sampling location		
	Yaba	Okobaba	Abule-Agege
SOD (U/mg/pro)	13.30 ± 1.02^a	25.30 ± 6.02^b	7.11 ± 2.82^c
CATALASE (mmol/min/mg protein)	1.09 ± 0.82^a	1.18 ± 2.21^a	1.86 ± 0.33^a
TBARS (nmol/min/mg protein)	121.90 ± 23.51^a	158.6 ± 27.78^a	221.4 ± 37.19^a
GSH (mmol / min/ mg protein)	5.20 ± 1.5^a	4.01 ± 2.41^a	13.12 ± 3.64^b
Protein (mg)	9.40 ± 4.2^a	7.11 ± 3.14^a	21.06 ± 7.40^a

(mean ± SD; n= 10)

3.2 Assessment of Oxidative Stress in *Sesarma huzardi*

The results of the biochemical enzymes activity are presented in Table 2. Examined samples of *S. huzardii* showed significant differences ($P<0.05$) in anti-oxidative stress enzyme activities and lipid peroxidation across sites. From the result, the lowest (7.11 ± 0.82 μ/mg protein) and highest values (25.30 ± 3.02 μ/mg protein) of SOD activity were obtained in samples of *S. huzardii* from Abule-Agege and Okobaba respectively. The SOD levels showed strong negative correlation with Cu and strong positive correlation with Fe and Zn ($P> 0.05$) across sampling sites but Cd and Pb showed weak negative correlation. With relatively higher mean values of 1.86 ± 0.33. mmol/mg protein recorded in samples obtained from Abule-Agege, there were no significant differences ($P < 0.05$) in observed Catalase enzyme activity (CAT) in muscle tissues of crab. The result of Lipid peroxidation in muscle tissues of *S. huzardii* expressed by the malondialdehyde levels (MDA/TBARS) was inconsistent among examined samples across all sites. But, relatively higher mean value of TBARS was recorded at Abule-Agege (221.4 ±17.189mmol/mg protein) as compared to the levels in crabs obtained from Yaba (121.90 ± mmol/mg protein). Similarly, reduced glutathione was highest in *S. huzardii* collected from Abule-Agege (13. 12 ± 3.64 units), while the lowest levels (4.01 ± 0.42 units) was recorded in crabs from Okobaba. Statistical analysis using the analysis of variance (ANOVA) showed no significant differences at $p > 0.05$ level of significance in the mean concentrations of TBARS values at both the control and polluted sites.

4. DISCUSSION

4.1 Heavy metal concentrations

Certain forms of metals have been shown to readily accumulate within crustacean tissues at much higher levels. Crab species mostly absorbed heavy metals from its feeding diets, sediments and surrounding waters resulting to their accumulation in reasonable amounts (McCarthy and Shugart, 1996). Generally, our results showed measurable but low concentrations of heavy metals amongst examined samples and across sites. The recorded metal contents in sediment in the

order: Fe > Zn > Cu > Mn > Pb and Cd differed significantly with highest mean concentrations recorded at locations closest to domestic and solid waste dumps. Furthermore, the accumulation patterns of trace metals found in tissues of *S. huzardii* collected from the Lagos lagoon mudflats showed that Cu was the most prevalent and abundant in tissues of crab while Pb was the least accumulated metal. The report of the present study is similar to the findings of previous studies on other contaminated ecosystems in Nigeria (Chukwu and Ogunmodede, 2005; Lawal-Are and Babaranti, 2014). It is important to note that metals/metalloids recorded at low levels in water and sediment could be higher in aquatic organisms through bio-concentration and bioaccumulation processes most times, resulting in progressively higher concentrations at higher trophic levels in the biota (Usese et al., 2017b). According to Elghobashy et al., (2001), bioaccumulation of heavy metals in crustaceans critically influences the growth rate, physiological and biochemical status. Furthermore, a number of deleterious effects including oxidative stress from heavy metals bioaccumulation in biological systems has been reported by some researchers (Farombi et al., 2007; Soundararajan et al., 2000).

Copper is an essential component for numerous oxidation reduction enzymes (cytochrome oxidase, uricase and tyrosinase). The toxicity of copper is reported to be dependent on the hardness and pH of water; being more toxic in soft water with low alkalinity (Taha, 2004; Chukwu and Odunzeh, 2006). On the other hand, Cd, a non-essential element with the potential for toxic effects was only detected at two of the sampling locations (Okobaba and site adjoining Abule-Agege creek). Zinc is also an essential micro nutrient required for normal growth and metabolic function for various crab species. Earlier studies have shown that Cd may replace Zn in certain enzymes, causing disease and severely limited oxygen metabolism of mitochondria in the liver of fish. Cadmium is also reported to accumulate in gills, liver and kidney of fish, causing damage to the gills and disturbing calcium balance (Wicklund et al., 1992). In addition, a previous study revealed that Zn can be toxic to crustacean at certain concentrations; causing mortality, growth retardation, tissues alternation, respiration and cardiac changes, inhibition of spawning, destruction of the gill epithelium and tissues hypoxia (Khalil et al., 2017).

4.2 Antioxidant defence system

Over the years, studies have shown that chemical analysis and biomarker assessment when combined, can offer more complete and biologically relevant information on the impact of pollutants on organism health (Martín-Díaz et al., 2005; Allan et al., 2006). Moreover, biomarkers, including antioxidant enzymes allows early detection of environmental problems (Vioque-Fernández et al., 2009; Bouraoui et al., 2010). In the present study, significant differences ($P<0.05$) was observed in anti-oxidative stress enzyme activities and lipid peroxidation across sites. Oxidative stress in aquatic organisms is induced by many chemical pollutants which may stimulate the production of reactive oxygen species and other oxygen free radical that can lead to alteration in antioxidant systems (Kadry et al., 2012; Valvanidis et al., 2006). Moreover, observed increase in antioxidant enzymes activities indicates adaptive responses of organism to counteract the oxidative effect of generated ROS or resistance to water pollutants toxicity against the damage caused by excessive amount of oxygen free radicals and oxidative stress (Gad, 2009; Carvalho et al., 2012). And SOD-CAT system is the primary defense mechanism against such oxidative stress. The recorded high and relatively low levels of SOD activity observed in crabs from Abule-Agege and Okobaba respectively suggest potential stress from those locations. SOD levels also showed strong negative correlation with Cu and strong positive correlation with Fe and Zn across sampling sites; but weak negative correlation was obtained for Cd and Pb. Meanwhile, there were no significant differences in observed Catalase enzyme activity (CAT) in muscle tissues of crab with relatively higher mean values at Abule-Agege. CAT detoxifies hydrogen peroxide to water and any elevated levels in organ may be associated with the hydrogen peroxide produced by SOD activity in the organism. In a related study, Achuba et al., (2014) reported increased CAT activity in *Heteroclarias* exposed to environmental pollutant while decreasing levels were however obtained in the different tissues of some other fish species. It has been noted that glutathione peroxidase catalyses the gluthathione dependent reduction of hydrogen peroxides which protects the oxidative damage of the erythrocytes due to lipid peroxidation (Khalil et al. 2017). In the present study, GSH levels exhibited strong positive correlation with concentrations of Cu, Fe and Zn in the tissues of *S. huzardii*.

Ahmad et al., (2000) also noted variations in the antioxidant response to oxidative stress in the various tissues of different species of fish. On the other hand, Kallil et al., (2017), reported that the concentrations of antioxidant enzymes (SOD, CAT, GPX, GST and GR) as well as oxidative stress biomarker (MDA) levels in organism tends to be temperature-dependents. Their study also showed that low temperature induced more free radical, leading to oxidative stress damage in tissues and produced more antioxidant enzymes to reduce the damaging effect of the free radicals

5. CONCLUSION

Chemical biomarkers can be used to evaluate the health status of brachyurans in an aquatic ecosystem. Additionally, antioxidant activity may be increased or decreased due to the presence of chemical stressors and depending on the intensity and duration of the stress, the tolerance of the exposed organism as well as their adaptation. The results of the present study provide information on metals bioaccumulation and the activities of enzymatic and non-enzymatic chemical biomarkers as useful indicator of pollution and the health status of the Hairy Mangrove Crab, *S. huzardii* from the study area.

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